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Exposure to Stressful Environments

Strategy of Adaptive Responses

Leon E. Farhi

The Hermann Rahn Laboratory for Environmental Physiology, Department of Physiology, State University of New York at Buffalo, Buffalo, N.Y., USA; Abteilung Physiologie, Max-Planck-Institut für experimentelle Medizin, Göttingen, FRG

Abstract. Any new natural environment may generate a number of stresses (such as hypoxia, water lack, and heat exposure), each of which can produce strains in more than a single organ system. Every strain may in turn stimulate the body to adapt in multiple ways. Nevertheless, a general strategy of the various adaptive responses emerges when the challenges are divided into three groups. The first category includes conditions that affect the supply of essential molecules, while the second is made up by those stresses that prevent the body from regulating properly the output of waste products, such as CO_2 and heat. In both classes, there is a small number of responses, similar in principle, regardless of the specific situation. The third unit is created by environments that disrupt body transport systems. Problems may arise when there is a conflict between two stresses requiring conflicting adaptive changes. An alternative to adaptation, creation of micro-environment, is often favored by the animal.

Survival of a population often hinges on its ability to occupy new ground. Relocation may be forced upon a species by a variety of causes, chief among which are natural disasters, gradual changes in the climate, displacement from the ecological niche by a dominant competitor, or the disappearance of some other animals or of plants upon which the group under consideration depends in one respect or another, usually as a food source. Spill-over into an adjacent territory also occurs when successful colonization creates an unacceptable increase in population density. Unfortunately for the migrators, contiguous areas frequently have different climatic features and therefore provide new living conditions, making existence syno-

Table I. Stresses produced by some environments

Environmental challenge	Stress				
	O_2 balance	heat balance	water balance	food supply	etc.
Altitude	+++	++	++	?	
Diving	+++	+++			
Desert life		+++	+++	+++	
Jungle life		+++			
Confinement	++	++	++	?	
Acceleration	+++				
Etc.					

A number of environments are listed in the leftmost column. The *major* stresses created by each are shown in the subsequent columns, with a gradation indicated by the number of + signs. 'Etc.' indicates that the table should be extended in both directions by adding environments and stresses.

nymous with the talent to adjust to altered, and often unfriendly, environments. The gamut of possible adaptive responses is extensive, covering subtle modifications in behavioral patterns at one end of the spectrum and major remodeling of anatomical or biochemical structures at the other extreme, with functional modifications making up the mid-range. Some of the adaptive changes may occur during the lifetime of a single individual, others require several generations.

In discussing the fundamentals of adaptation, Dejours [1] defined this process as 'a change minimizing the physiological strain which results from a stressful environment'. The chain of events can therefore be summarized by the following progression:

New environment → stress → strain → adaptation.

The scientist who works at the bench is usually interested in a single cause-consequence pair, studying an isolated variable under carefully controlled and reproducible conditions, and therefore has no problem with this scheme. His colleague in the field who attempts to provide real-life synthesis of laboratory findings is in a far less favorable position because this deceptively simple sequence is complicated by the fact that each of the steps is multiplicative: an environment often creates more than one stress, each of

Table II. Strains caused by environmental stresses

Stress	Strain				
	circulatory system	respiratory system	urinary system	digestive system	etc.
O_2 balance	+++	+++		+	
Heat balance	+++	+++			
Water balance	+++	+	+++	++	
Etc.					

Major strains produced by each stress are shown, with the gradation indicated by the number of + signs. 'Etc.' extends the table in both directions.

which – if severe enough – may generate several strains, and any one of these usually triggers numerous adaptive mechanisms. Table I describes the stresses that are caused by selected environments while table II lists the strains that correspond to some of these stresses.

Because the plethora of relationships shown in tables I and II would make a list of environments and adaptive steps both lengthy and unrewarding, it is necessary to resort to a simpler approach, and to attempt to sketch adaptation in broader lines, focusing on salient points only. For a more comprehensive, up-to-date review, see Senay [2].

The 'milieu exterieur' acts both as a source and as a sink, supplying the organism with its basic requirements such as metabolites, water and oxygen, and accepting in return carbon dioxide and other wastes, including heat. Thus, animals have to face two major types of environmental problems: on the 'input' side there can be a mismatch of supply to demand, while on the 'output' side, the surrounding medium may not be able to handle breakdown products, forcing the body to store them. Let us examine both sets of challenges in more detail.

Environments Causing 'Input' Problems

The common adaptive strategies that one can detect are those with which we are familiar in the field of economics; when availability fails to meet demand one can reduce consumption, switch to alternate sources or

develop new routes of supply, rely on substitutes, improve internal transportation, and prioritize distribution so as to ensure that vital operations are maintained. If the problem is recurrent and can therefore be anticipated, one should stockpile essential materials before the emergency occurs. The way in which living organisms adopt these measures when they are subjected to stressful environments can be illustrated by what is intended to be a selection of appropriate examples rather than an exhaustive list.

Lack of Metabolites: Complete or Partial Starvation

Adaptation to a limited caloric intake triggers a number of the responses we have just reviewed. There is a decrease in metabolism, which has the obvious consequence of stretching the insufficient supplies. This drop in demand, reviewed by Grande [3] for man and animals can be attributed to two causes: (1) body mass shrinks, reducing the size of the body to be fed, and (2) the metabolic rate of active tissue slows down gradually, stabilizing after a few weeks. The first of these two changes cannot be considered to be adaptive; the second certainly is. In parallel, the organism develops alternate sources of essential materials, an example being provided by generation of glucose from other entities, in particular lipids. Storage of fat before an extended period of fasting has been well documented in hibernators and in birds preparing to migrate, and is discussed in terms of the incubating penguin by Le Maho [4] elsewhere in this volume.

Water Balance

Whereas some natural stresses (such as problems of O_2 supply and of insufficient food intake) can occur on their own, i.e. in the absence of other perturbing factors, this is seldom the case for water balance. Lack of water is usually associated with thermoregulatory problems, and occurs not only in very hot environments but also in extremely cold ones, where the energy needed to melt ice makes water in liquid form a scarce commodity. Furthermore, these two conditions may boost water loss, the first through sweating and/or increased insensitive water loss, the second mainly by decreasing the water vapor content of the inspired air.

Water economy can be achieved only by reducing outgo. Desert rodents produce feces which are practically devoid of water, urine becomes more concentrated, sweating is reduced. A number of steps can also be taken to diminish pulmonary water vapor loss, either by having it deposit in the upper airway during expiration and entraining it during inspiration, or simply by reducing minute volume. Clearly, a sizeable drop in ventilation is

tolerable only if it is coupled with a shift of the hemoglobin dissociation curve to the left, so as to maintain adequate oxygenation of the arterial blood.

Much has been made of the ability of the kangaroo rat to obtain water from metabolic sources, allowing the animal to live without intake of preformed water. It has been pointed out [5] that H_2O extraction from food is more complex than would appear and can be successful only under certain conditions: one can calculate that as the camel uses its fat reserves, it also obtains water but the increased O_2 requirement implies the need for extra ventilation and hence a rise in respiratory water loss. The latter may exceed the gain, leaving the animal with a water deficit!

It is well known that animals can carry water reserves. In addition, the behavior of some desert rodents generates an extracorporeal water store: by hoarding grain in their burrows, where it absorbs some of the moisture [5], they recycle part of the water they lose.

Oxygen Balance

Adaptation to an inadequate supply of oxygen deserves special mention for several reasons, the most important of which is probably that the body's O_2 stores are woefully small in comparison to its needs. While life without water or metabolites is possible for variable but extended periods of time, the total amount of oxygen stored in the body and available for emergency use can last only a few minutes at rest, much less at exercise.

Another unusual feature of this stress is that there is usually no shortage (or excess) in the *amount* of O_2 available, as is the case for water or food. Even at the top of Mount Everest, a climber would have an infinite number of oxygen molecules at his disposal; his problem is that this gas is presented at a low partial pressure, which hinders its transport.

Hypoxia

Decrease in partial pressure of inspired O_2 can be caused by one of two conditions: there may be either a drop in the fractional concentration of the gas in the environment, as occurs in burrows, or a reduction in total barometric pressure, as is found at altitude. The usual adaptive response is multifaceted, and some of its features depend on whether one is looking at a single acute episode, repetitive encounters with the stress, or chronic exposure. Just as is the case with starvation, a severe deficit in oxygenation leads to a reduction in consumption [6], but the strategy relies mainly on lowering the ventilatory and the circulatory resistances to oxygen transport so as to

decrease the O_2 pressure drop at each step of the oxygen cascade. As a result of these changes, the total P_{O_2} difference between inspired and mean end-capillary blood – to the extent to which this is reflected by mixed venous blood – is reduced from more than 100 Torr at sea level to one-third of that value or less at high altitude. Note that whereas the ventilatory conductance can only be improved by increasing alveolar gas flow, the rise in circulatory conductance is due to both a quantitative factor, i.e. a boost in cardiac output, and a qualitative one, resulting from higher hemoglobin concentration and often a favorable displacement of the hemoglobin dissociation curve, this shift being particularly well illustrated in fish that breathe O_2-poor water. When hypoxia is extreme or is combined with exercise, which increases the O_2 demand, another mechanism listed earlier, i.e. switch to a different metabolic pathway, is often invoked as the subject or animal satisfies part of the metabolic demand through anaerobic glycolysis. Clearly, this can take place for only a limited time, after which oxygen must be used to recycle lactate. For a more extensive discussion of the combined effects of hypoxia and exercise, see Cerretelli [7].

Some of the other general strategies which we have listed earlier are also implemented in specific cases of adaptation to low oxygen. As examples (1) a number of species have undergone evolutional changes to survive in the presence of hypoxia: certain fish living in oxygen-poor water have developed lungs of various degrees of complexity, often using them as an auxiliary system, i.e. only when the water cannot supply the O_2 demand [8]; (2) diving mammals illustrate the benefits to be gained by prioritizing blood flow distribution and hence oxygen delivery: severe vasoconstriction of all the beds that subserve areas whose continuous unimpeded function is not imperative allows the animals to allocate the scarce oxygen reserves practically entirely to their heart and their brain; and (3) the blood volume of these diving mammals and their hemoglobin concentration are both high, increasing the amount of O_2 that can be stored prior to the dive [9].

Hyperoxia

At the cellular level, there are chemical reactions that scavenge superoxide and associated products, as reviewed by Crapo [10]. In contrast to the rich, assorted spectrum of systemic reactions to low oxygen, adaptive responses to hyperoxia appear to be limited to vasoconstriction and a moderate decrease in cardiac output, which reduces O_2 transport to the tissues, moderating the rise in cellular P_{O_2}. The input that triggers that

Table III. Adaptive responses to 'input' environmental stresses

Response	Stress				
	lack of food	lack of water	hypoxia	hyper-oxia	etc.
Decreased consumption	+++	+++	++		
Development of alternate sources/supply routes		++	++		
Substitution	++		+		
Transport optimization			++++	+	
Allocation of priorities	+	++	+++		
Stockpiling	++	+	++		

Only the most frequently used responses are shown. For details, see text.

response is not clear, although some experiments [11] indicate that the phenomenon is dependent, at least in part, on the P_{O_2} beyond the arterial tree.

One should note that, as opposed to hypoxia, which occurs in a variety of natural environments, hyperoxia is almost invariably an artificial challenge. It does occur in fish living in ponds or seawater pools where the photosynthesis of a rich aquatic flora creates diurnal P_{O_2} cycles with a peak that can reach 600 Torr [12], but not in air breathers. The existence of adaptive mechanisms, limited as they may be, is either fortuitous or the consequence of the fact that, as life's environment went from a reducing atmosphere to an oxidizing one, some responses against the increasing O_2 level may have emerged. Obviously, this explanation is tenable only for the cellular reactions, since complex organisms having a well-defined circulatory system developed long after oxygen had become part of our environment.

Overview of Responses

The parallelism of the various steps is emphasized in table III, which lists the most common features encountered in the environmental stresses we have just sketched. Because of space limitations, several challenges have been grouped under one heading. As an example, 'Hypoxia' covers such diverse conditions as the drop in P_{O_2} encountered at altitude or by fish living

in waters with little oxygen, the decrease in oxygen fraction faced by the fossorial species, or the temporary lack of oxygen of an animal during a breathhold dive. Obviously, only some of the responses listed in one column will be elicited by one specific environment.

Environments Causing 'Output' Problems

With rare exceptions, problems on the 'input' side are caused by deficiency rather than by excess, since in the vast majority of cases an animal cannot be forced to avail itself of an oversupply but remains free to limit its intake to a desirable, safe amount. On the 'output' side, the stress may be either surfeit or scarcity: complete elimination of some of the important products of metabolism is often undesirable, and since many of these (such as heat and carbon dioxide output) have to be adjusted to maintain body levels within a narrow range, an abnormally high loss can be as close to catastrophic as reduced elimination.

Responses to environmental conditions that affect output are perhaps best understood in terms of a simple electrical model. In this analog, the material to be eliminated is compared to electrons moving from a high potential at the point where they are generated, to a low potential in the surrounding medium, through a series of resistors, each of which represents a step in the transport chain. The desirable level at each location – equivalent to voltage – is set by adjusting properly the different resistances. Environmental stresses may act on the system either by altering the potential of the sink or by affecting the resistance of the outermost transport link(s). It is easy to recognize immediately the possibility of three adaptive responses, namely (1) readjustment of the resistances in the transport chain; (2) change of the intensity of the current generated at the source; and (3) creation or enhancement of capacitances to better handle retention, in particular when this occurs in surges. We shall use stresses on the thermoregulatory system and on CO_2 elimination to illustrate these mechanisms.

Thermoregulation

Exposure to Heat

It is obvious that an increase in ambient temperature causes an immediate drop in the temperature gradient across the skin, which means that less heat can be dissipated if skin conductance remains fixed. The obvious

compensation, i.e., a decrease in the resistance to heat transfer across the outer insulation layers, is brought about mainly by vasodilation of the skin and the underlying tissues. At the same time, an attempt is made to cool the contact surface by evaporative heat loss, the efficiency of sweating depending of course on the ambient humidity. The problem is that these responses require an increase in peripheral perfusion, easily accomplished at rest, but not so at exercise, because (1) the skin competes with muscle for available blood flow, and (2) sweating decreases plasma volume and may therefore interfere with development of maximal cardiac output. Thus, core temperature rises during exercise, restoring (at least partly) the outward heat gradient. Interestingly, the acclimatized subject is presented with an additional problem: as he sweats more, he becomes dehydrated (and should compromise his maximum cardiac output) faster; some desert animals have risen to the challenge by developing mechanisms that allow them to maintain plasma volume while subjected to heat dehydration. For details, see the chapter by Horowitz and Samueloff [13].

Exposure to Cold

Cold stresses are generated not only when the body is exposed to a lower ambient temperature, but also when body heat is drained at too fast a rate because of an increase in thermal capacity or conductance of the surrounding medium. Not surprisingly, adaptation to cold invokes the same regulatory mechanisms as heat exposure, but in the opposite direction. When the stress is essentially continuous, as in the case of diving mammals, physical insulation is provided by a thick layer of subcutaneous fat. When the exposure is transient, but repeated, resistance to heat flow is increased by peripheral vasoconstriction, in particular in acclimatized individuals, whose maximum tissue insulation exceeds that of nonacclimatized subjects having the same cutaneous thickness [14]. Metabolism rises, generating more heat, and can of course be boosted dramatically by shivering [15].

Carbon Dioxide Retention

The first and foremost defense mechanism, increase of the CO_2 conductance, that is, hyperventilation, is brought into play whenever possible. Storage of the retained CO_2 is also attempted, and in this respect one must distinguish between animals that are faced continuously with high levels of CO_2, such as rodents living in burrows, and those that are exposed only intermittently. In either case the problem is to maintain pH, but this is accomplished by different means. The animals that live in a high CO_2

Table IV. Adaptive responses to 'output' environmental stresses

Response	Stress			
	heat	cold	CO_2 retention	etc.
Resistances adjustment	+++	+++	+++	
Flux adjustment	+++	+++		
Increased capacitance			+	

For details, see text.

environment retain bicarbonate in their plasma and extracellular fluid; on the other hand, diving mammals rely on better buffering power of their blood.

Overview

Table IV shows the similarity between the various mechanisms just listed. The reservations expressed in relation to table III apply here also.

Environment and Transport Problems

The preceding two sections have made it clear that optimization of the internal transport system is often the critical feature of adaptation. It is therefore appropriate to indicate here that certain environments leave the input and output sides unaltered but disrupt the ability of the body to either supply the cell or detoxify it. One such condition is exposure to high pressure, where the rise in respired gas density makes it difficult to maintain adequate alveolar ventilation. Another is increased acceleration, especially along the long axis of the body, that is foot-to-head. This stress, which is equivalent to an increase in gravitational pull, is always manmade, but is nonetheless interesting from both the scientific and the practical points of view. It acts primarily by magnifying the normal tendency of the blood to pool in the dependent parts of both the systemic and pulmonary circulations, thus reducing venous return and hence cardiac output. This sequence often lowers brain perfusion to dangerous levels or even reduces it to zero,

causing the dreaded 'black-out' of military pilots, who may be subjected to accelerations of more than 10 G.

Duration of exposure to high G is usually much too short to evoke adaptive mechanisms, but one must remember that the strain depends on the pressure exerted within the vessels in the dependent parts of the body, a pressure that is a function not only of gravity, but also of the height of the blood column. Therefore, very tall animals have to defend themselves against edema formation in the lower part of their limbs even at normal G. The complete picture of the adaptive mechanisms is not yet clear, but it is known that the skin of the giraffe's extremities is very tight and can therefore act as a natural anti-G suit.

The topic of acceleration fits perhaps best within the scope of this review when one considers the opposite end of the scale. i.e. microgravity, a situation in which the effects of gravity are counterbalanced to a greater or lesser extent. The best known example is that of astronauts in orbit, in whom centrifugal and gravitational accelerations are equal and opposite. Alternatively, during immersion, buoyancy compensates for gravity in the systemic circulation, while recumbency minimizes the length of the body axis on which gravity operates; these two types of challenge are often used as a 'poor man's zero G simulation', and have demonstrated that if the exposure is long enough, some of the adaptive mechanisms that have been developed in response to our normal 1G environment may be blunted or even disappear, a phenomenon known as deconditioning. The reasons and time course of this process are reviewed by Pendergast et al. [16] in this volume.

Adaptation: Problems and Alternatives

From what has been said so far, it is fairly clear that most environmental stresses can be minimized by the numerous adaptive responses that they trigger; we must balance the picture by indicating that the reactions themselves may not be free of problems. A conflict will arise when two different stresses, caused by the same environment, call for opposite responses. This divergence is sometimes obvious: a subject exposed to altitude must often respond to both hypoxia and water intake limitations, but whereas the former calls for hyperventilation, water conservation demands a decrease in respiratory water loss. At other times, a beneficial reaction fails to take place, and we are left with the speculation that it may be opposed by a stress and a counterstimulus that we do not see. Such is the case in the response to

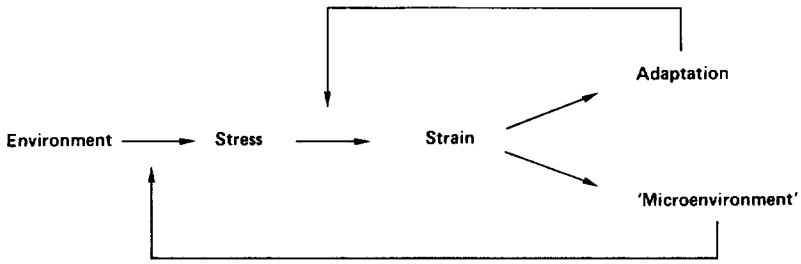

Fig. 1. Possible responses to new environments. As defined by Dejours [1], adaptive mechanisms minimize the strains caused by a stressful environment. The alternative, creation of a 'micro-environment', allows the animal to avoid or minimize some of these stresses. Such a strategy relies more on behavior than on physiological changes.

exposure to altitude, where, in spite of the increased pulmonary arterial pressure, distribution of ventilation/perfusion ratios is not improved. This has led Kreuzer et al. [17] to conclude: 'It is of great interest why such a potentially effective defense against hypoxia as a reduction in oxygen gradient should be excluded from the acclimatization process. There is no direct evidence to explain this, but in all probability the exclusion is obligatory as a result of other adaptive mechanisms.' Finally, at times, it is the adaptive response itself which creates an additional stress, as exemplified by dehydration occuring as a result of response to a heat load. Another important consideration is that long-range adaptive processes triggered by one environment may make it more difficult to respond to another stressful situation.

If adaptation to environmental stresses presents serious problems, is there an adequate substitute? Let us examine briefly two alternatives. The first is simply an increased tolerance to changes in physiological status. Along these lines, one can think of the Australian aborigine whose temperature drops during the night and of the camel who stores heat during the day and loses it during the cool desert night.

Many species have attacked the problem in a different fashion: by creating a micro-environment in which to live, one reduces or abolishes the stress and thus the need to minimize the strain (fig. 1). This micro-environment may be communal like the beaver's log house and man's climate-controlled housing, or individual like the eskimo's suit that provides its owner with semitropical conditions. Where at all possible, this strategy seems superior because it does not require changes in structure or in

physiological function and therefore ensures species survival without sacrificing flexibility. One should note, however, that in making their own 'milieu-demi-exterieur', animals often trade one stress for another. That they choose to do so raises the tantalizing question of hierarchy of stresses, a fruitful field for further investigation.

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Leon E. Farhi, MD, Department of Physiology, SUNY at Buffalo, Farber Hall, Buffalo, NY 14214 (USA)

Exercise Effects on the Size and Metabolic Properties of Soleus Fibers in Hindlimb-Suspended Rats

SCOT C. GRAHAM, B.S., ROLAND R. ROY, Ph.D., STEVE P. WEST, B.S., DON THOMASON, Ph.D., and KENNETH M. BALDWIN, Ph.D.

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The soleus atrophies rapidly when a rat is subjected to hindlimb suspension (HS), probably as a result of a decrease in the force encountered by the muscle. To test this premise, adult female rats were HS and half the rats were exercised (HS-EX) on a treadmill for 1.5 h · d^{-1} at 20 m · min^{-1} and a 30% grade. After 4 weeks, the midbelly of the soleus was prepared for histochemical analysis. Fibers were typed as dark or light staining for myosin ATPase, alkaline preincubation. Fiber size and quantitative histochemical enzyme activities of succinate dehydrogenase (SDH) were determined using a computer enhanced image processing system. In comparison to age-matched controls, the soleus wet weight was 69 and 30% smaller in HS and HS-EX rats. The mean cross sectional area of the dark ATPase fibers was reduced by 46 and 18% and light ATPase fibers by 69 and 48% in the HS and HS-EX, respectively. The percent dark ATPase fibers increased from 10% in the control rats to 19 and 17% in the HS and HS-EX. In both suspended groups, SDH activities in light ATPase fibers were 40% higher than control. The SDH activity of the dark ATPase fibers of HS-EX was 20% higher than control, while the dark ATPase fibers of HS were similar to control. To determine the degree to which these increases in SDH could be related to reductions in fiber size rather than increases in the actual amount of protein, integrated activity (activity/min x area) was calculated per fiber. The integrated activity of SDH suggests a net loss of this enzyme in the light and dark ATPase fibers of HS, but only in the light ATPase fibers of the HS-EX rats. In summary, daily treadmill exercise ameliorated, but did not prevent, the muscle fiber atrophy induced by HS in a slow extensor muscle. Further, SDH activity per fiber was maintained or elevated in the HS and HS-EX rats regardless of the variable changes in fiber size induced by suspension and endurance type exercise.

From the Neuromuscular Research Laboratory, and the Brain Research Institute, University of California, Los Angeles, California; and Department of Physiology and Biophysics, University of California, Irvine, California.

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Address reprint requests to Dr. Roland R. Roy, Brain Research Institute, Center for Health Sciences, UCLA, Los Angeles, CA 90024.

IT IS APPARENT that the forces encountered during everyday situations are critical in the maintenance of normal skeletal muscle properties, especially for extensor muscles. When these forces are no longer present, the affected muscles exhibit characteristic changes. Generally, the muscles atrophy and demonstrate shifts in their mechanical properties towards those found in "faster" muscles. This has been shown to be true in several models which are assumed to induce dramatic decreases in *in vivo* muscle force production, such as spinal isolation (26), spinal transection (2,27), limb immobilization (34), hindlimb suspension (9,18,31,35), and spaceflight (10,15,19). It also appears that not all muscles are affected equally by the removal of these ground reactive forces. For example, the antigravity muscles of the lower leg, the extensors (e.g., the soleus and medial gastrocnemius) are affected to a greater degree than are the flexors (e.g., the tibialis anterior and extensor digitorum longus) (9,12,22,23,32,35). It also has been shown that within a functional group of muscles, the "slower" muscles, i.e., those muscles which have a relatively high proportion of fibers that stain lightly for myosin ATPase, alkaline preincubation, are more responsive to the removal of force than muscles which have a relatively high proportion of darkly staining fibers (24,35). In this light, the soleus, a predominantly slow extensor muscle, appears to be a good model to study the effects of weightlessness or unloading on skeletal muscle.

Given the apparent importance of muscle loading in maintaining normal muscle properties, particularly in the extensors, it is likely that exercise can be used to attenuate the functional decrements associated with chronic decreases in muscle force. For example, Roy *et al.* (27) have shown that assisted treadmill walking for 30 min · d^{-1} results in a significant reduction in the atrophy and associated decrement in force capabilities of the soleus muscle resulting from chronic, low thoracic spinal cord transection in cats. Similarly, several recent, mainly preliminary, reports (12,22,30,33) have

suggested that treadmill exercise of various durations and intensities may help to ameliorate the atrophic response associated with hindlimb suspension (HS). Other forms of loading, such as grid climbing with attached weights (13) and chronic stretch of the hindlimb muscles (18) in HS rats also have been shown to benefit the affected muscles. Thus, the purposes of the present study were to 1) evaluate the morphologic and succinate dehydrogenase (SDH) adaptations occurring in soleus fibers identified by myosin ATPase type when unloaded for a period of 4 weeks, and 2) assess the effectiveness of an endurance exercise program in ameliorating these changes.

METHODS

Experimental design and suspension procedures: Adult female Sprague-Dawley rats (approximately 275 g, body weight, 8 weeks of age) were obtained from Taconic Farms, Germantown, NY. The rats were assigned randomly to one of three groups: sedentary control (CON, n = 8), hindlimb-suspended (HS, n = 8) or hindlimb-suspended plus exercise (HS-EX, n = 8). Rats from each group were housed in identical cages and maintained in a light and temperature controlled environment. Food and water were provided *ad libitum*. Both HS groups were subjected to a suspension period of 28 d. The suspension model used has been described in detail (32,33). Briefly, the rats were suspended by the application of a tail cast traction bandage covering less than one-half the tail surface and thus allowing adequate thermoregulation through the tail. The tail cast was attached to a swivel hook mounted at the top of the cage, allowing free 360° rotation. The height of each animal was adjusted to allow the rat to support its weight and to move about freely on its forelimbs while the hindlimbs were elevated to prevent contact with the floor or the sides of the cage. Animals were checked daily for signs of tail lesions or discoloring, unusual breathing patterns, or undue discomfort. An animal showing any of these signs was immediately removed from the study.

Rats in the HS-EX group were exercised on a treadmill with their tail casts attached to the treadmill cover rails to prevent them from dragging. Initially, the rats were run for 10 min daily with 5 min added each day until they were running 1 $h \cdot d^{-1}$. Subsequently, the running period was increased by 10 $min \cdot d^{-1}$ until a final bout of 1.5 $h \cdot d^{-1}$ was attained. The exercise was performed at a speed of 20 $m \cdot min^{-1}$ and at a 30% grade.

Tissue preparation: Following 28 d of HS, the rats were anesthetized with sodium pentobarbital (50 $mg \cdot kg^{-1}$, i.p.) and the soleus, along with other muscles, and the adrenal glands were removed. The tissues were rinsed with cold saline, trimmed of excess fat and connective tissue, blotted dry, and wet weighed. Subsequently, the rats were sacrificed by exsanguination. A 5–10 mm thick cross section was taken from the midbelly of the soleus, mounted on cork in a manner such that the fibers were perpendicular to the cork surface. The sections were quick-frozen in isopentane cooled to −160°C by liquid nitrogen. This process was completed within 15 min after removal from the animal.

The tissue was cut in 10-μm thick serial sections in a cryostat maintained at a temperature of −20°C and mounted on coverslips. Sections then were stained qualitatively for myosin adenosine triphosphatase (myosin ATPase, alkaline preincubation, pH = 8.8, and acidic preincubation, pH = 4.35) as described by Nwoye *et al.* (21). Although the assumption is generally made that fast-twitch fibers stain darkly and slow-twitch fibers stain lightly at an alkaline preincubation, this is unlikely to be true in a strict sense because in many cases there is not a clear separation of the physiological properties into two populations of motor unit types. However, this staining property does provide a consistent means of separating fibers according to the sensitivity of myosin ATPase activity to pH. Quantitative histochemical methods to determine SDH activities have been published recently (11,12,20,24). Briefly, the activity of SDH was determined in a medium containing 100 mM phosphate buffer (pH 7.6), 10 μM sodium azide, 20 μM 1-methoxyphenazine methylsulfate, 1.5 mM nitro blue tetrazolium, 5 mM ethylenediaminetetraacetic acid (disodium salt) and 48 mM succinate (disodium salt). The reaction was run at room temperature in the dark, and stopped at 8 min with repeated washings of distilled water. All sections were air dried, mounted with Aq mount and stored on an image processing system within 6 h. The quantitative histochemical assay for SDH has been found to be linear up to 12 min (20). The reactions were terminated prior to this point, thus the optical density per min readings (see below) represented a steady-state enzymatic reaction.

Tissue analysis: One representative sample of fibers from each muscle was chosen for analysis. In order to remain consistent, a region located at a particular site (i.e., based on anatomical features) in the cross section and containing a mixture of fiber types was selected. Four pairs of alternating sections were cut for the quantitative SDH staining reactions. One section in each pair was incubated in a medium either containing or lacking substrate. Sections incubated without substrate acted as tissue blanks to correct for nonspecific staining. The final optical density for each fiber was equal to the mean value obtained from sections with substrate minus the value of the tissue blanks lacking substrate. The quantitative histochemical processes have been verified biochemically with respect to tissue thickness and time of incubation (20). In addition, only a 3.3% coefficient of variation was observed in the protein analyses of 15 serial sections cut at a 10-μm thickness. These data indicate that the optical density readings are directly related to the volume of muscle tissue in the sections.

Tissue sections were digitized on a computer enhanced image processing system and stored on magnetic tape as described previously (11,12,20,24). Briefly, this system is composed of a light microscope (Zeiss M14), an image-digitizing television system (Eye Com II, Spatial Data System model 108PT) with a high-speed arithmetic unit, a line printer (Printronix), a magnetic tape drive (Kennedy model 9110) and a minicomputer (PDP-11/34) with dual-platter disc drive. An array of picture elements (640 × 480 pixels) are quantified to 256 grey levels that are then converted automatically to optical density. An operator-controlled joystick allows

objects to be defined and analyzed independently. The camera is calibrated for light intensity with monochromatic light-interference filters. The camera's dark current is taken to be equivalent to 0% transmission of light and its saturation point is at 100% transmission. The optical density range is from 0.002 to 2.0. All light with a wavelength <450 nm in each density measurement is eliminated with a cut-off filter.

Approximately 25-60 adjacent fibers were analyzed from each section. These fibers were typed as darkly or lightly staining for myosin ATPase based on their optical density. Subsequently, fiber cross-sectional area (CSA) was determined and the reaction products from the SDH histochemical reaction were quantified. Interrelationships between fiber size and enzyme activities were determined from these samples. Care was taken to select fibers which appeared to be cut perpendicular to their longitudinal axis. Also, fibers that had freezing artifact or appeared to be degenerative were not analyzed quantitatively. The percentage of dark and light ATPase fibers of each muscle was determined by qualitatively typing between 1,600–2,400 fibers in each muscle.

Statistical procedures: A procedure was used which allowed for an unequal number of observations. Significant differences ($p < 0.05$) between groups were determined using a nested one-way analysis of variance. The Bonferroni (Dunn) *t*-test was used for determining significant differences between the three groups and consisted of performing three unpaired *t*-tests using the separate estimate of common variances and an adjusted significance level of $p < 0.017$ (i.e., 0.05/3).

RESULTS

Body, muscle, and adrenal weights: Following 28 d of HS, the mean body weight of the HS group was significantly smaller than that of the CON group (Table I). In contrast, the HS-EX mean body weight was similar to CON and significantly larger than HS. Soleus wet weights were approximately 70 and 30% smaller in HS and HS-EX when compared to CON. Adrenal weights were not significantly increased due to the suspension protocol but were over 50% larger in the HS-EX in comparison to CON. These data suggest that the exercise regime, in combination with the suspension, was stressful for the rats.

Fiber type and size: The muscles from CON rats had 10 ± 2% fibers staining darkly for myosin ATPase at an alkaline preincubation (Table I). Suspension resulted in almost a doubling in the number of darkly staining fibers (i.e., from 10 to 19%), although the large inter-animal variability resulted in no statistically significant difference between the two group means. The HS-EX group had a percentage of darkly staining fibers that was between the CON and HS values.

In the CON group, the light ATPase fibers were 42% larger than the dark ATPase fibers (Fig. 1), a relationship consistent with previous data (11,12). The CSA of the light ATPase fibers in both suspended groups were significantly smaller than CON, i.e., 69 and 48% in HS and HS-EX, respectively. In contrast, the dark ATPase fibers atrophied by 46% in the HS, but only 18% in the HS-EX rats. As a consequence of this differential atrophy, the two fiber types became similar in size following 28 days of HS (Fig. 1).

The frequency distributions of CSA for both fiber types are illustrated in Fig. 2. In the CON group, the range of CSA in the light ATPase fibers is considerably greater than in the dark ATPase fibers (Fig. 2) and the mean CSA of the light ATPase fibers is approximately 50% higher than that of the dark ATPase fibers (Fig. 1). In the HS group, however, both the range and mean CSA are almost identical for the two fiber types. This observation emphasizes the greater relative and absolute atrophy of the light in comparison to the dark ATPase fibers following suspension (Fig. 1). The daily exercise regime partly prevented the shift towards smaller fiber sizes, particularly in the dark ATPase fibers where some fibers actually may have hypertrophied (Fig. 2).

Single fiber enzyme activities: Following 28 d of HS, the mean fiber SDH activity of the light ATPase fibers was 40% higher compared to CON ($p > 0.05$), while that

TABLE I. BODY, MUSCLE, AND ADRENAL WEIGHTS, AND PERCENT DARK ATPASE FIBERS IN CONTROL (CON), 28-D HINDLIMB-SUSPENDED (HS), AND 28-D HINDLIMB-SUSPENDED PLUS EXERCISE (HS-EX) RATS.

	CON (8)	HS (8)	HS-EX (8)
Body Weight (g)	295 ± 6	265 ± 7*	285 ± 7^{+}
Soleus Weight (mg)	107 ± 5	33 ± 3*	75 ± 4*$^{+}$
Adrenal Weight (mg)	79 ± 5	83 ± 10	120 ± 4*$^{+}$
Dark ATPase Fibers (%)	10 ± 2	19 ± 2	17 ± 4

Values are means ± standard error of the means. The number of rats in each group is given in parentheses. *Denotes a significant difference between CON and HS or HS-EX. $^{+}$Denotes a significant difference between HS and HS-EX.

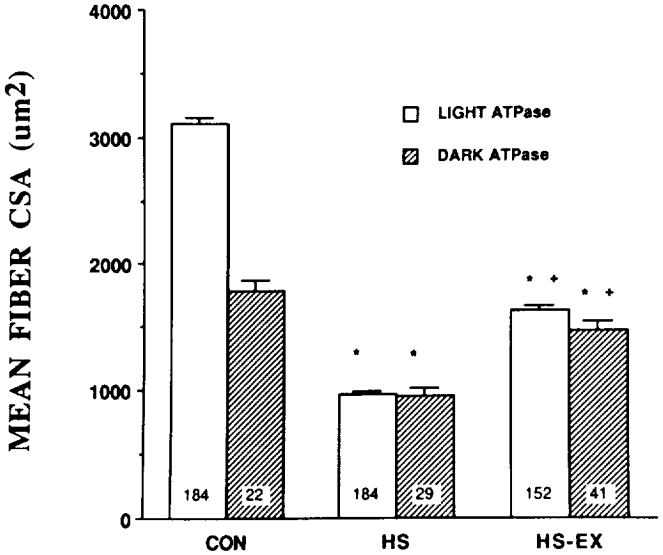

Fig. 1. Mean fiber cross-sectional area (CSA, μm²) of light and dark ATPase fibers of the soleus of control (CON), and 28-d hindlimb suspended (HS) and HS plus exercise (HS-EX) rats. Number of fibers is indicated within each bar. Vertical bars are standard errors of the mean. *Significant difference between CON and either HS or HS-EX. †Significant difference between HS and HS-EX. p = 0.05.

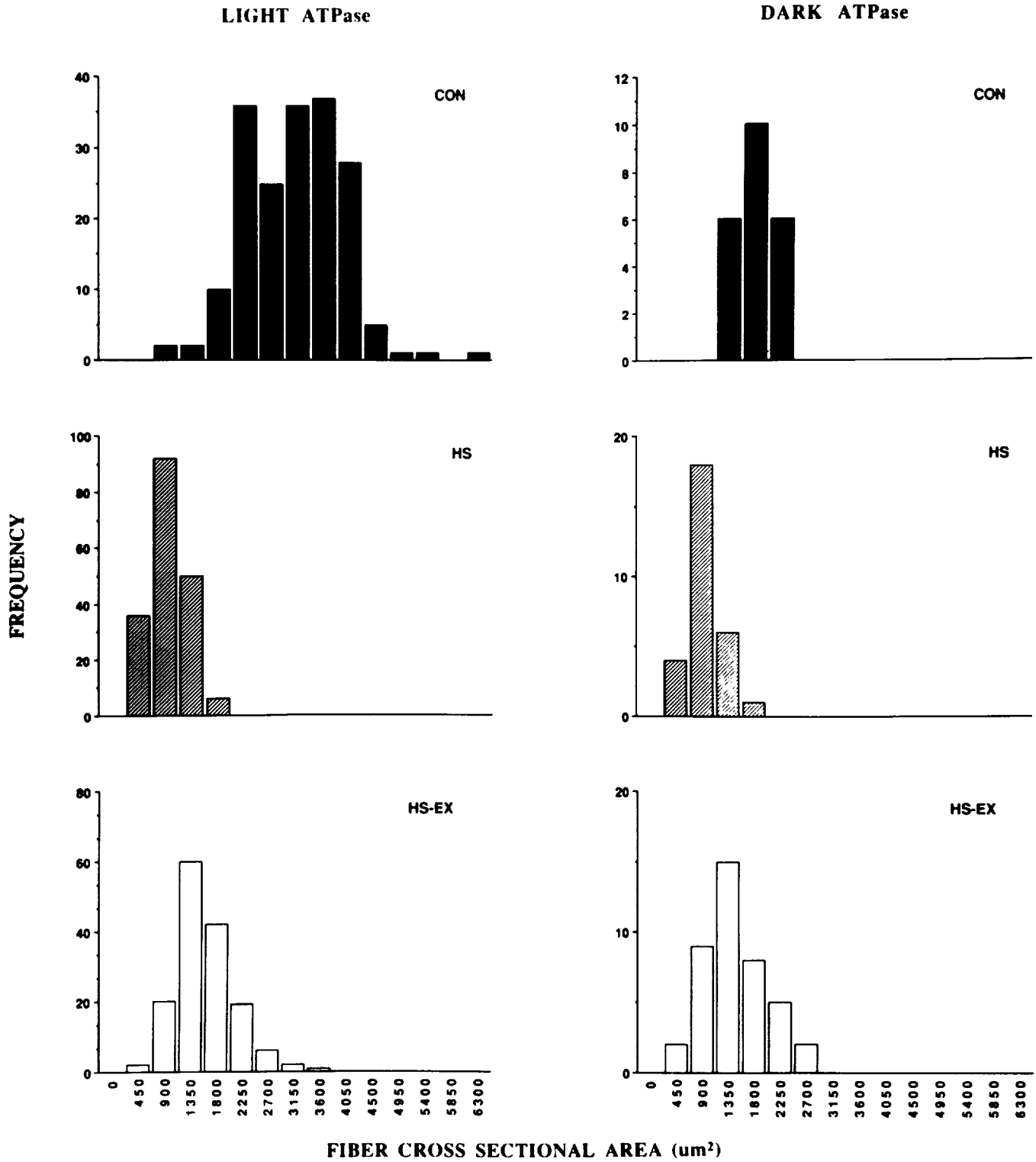

Fig. 2. Frequency distributions of fiber cross-sectional areas for light and dark ATPase fibers of the soleus of control (CON), and 28-d hindlimb suspended (HS) and HS plus exercise (HS-EX) rats.

of the dark ATPase fibers was similar to CON (Table II). Exercise potentiated the SDH activity in the dark, but not the light, ATPase fibers. The light ATPase fibers had a smaller range in SDH activities than the dark ATPase fibers in the CON group (Fig. 3). In all three groups, the light ATPase fibers had a smaller minimum SDH activity level than the dark ATPase fibers in their respective groups. In both fiber types and in both HS groups, there was an increase in the proportion of fibers having SDH activities in the upper range of values (Fig. 3). However, the range of values at the lower end of the distribution was unaffected. Thus, it appears that only some of the fibers were affected by suspension. Overall, in each group, the dark ATPase fibers had a higher ($p >$

TABLE II. SUCCINATE DEHYDROGENASE (SDH) ACTIVITY AND INTEGRATED SDH ACTIVITY (ISDH) OF SOLEUS MUSCLE FIBERS IN CONTROL (CON), 28-D HINDLIMB SUSPENDED (HS), AND 28-D HINDLIMB SUSPENDED PLUS EXERCISE (HS-EX) RATS.

	CON	HS	HS-EX
SDH (OD/min × 10^{-4})			
Light ATPase Fibers	42.77 ± 1.28 (164)	59.97 ± 2.56 (184)	59.76 ± 2.35 (152)
Dark ATPase Fibers	70.80 ± 6.11 (20)	78.83 ± 5.34 (29)	84.66 ± 5.02 (41)
ISDH [SDH activity (OD/min) × CSA (μm^2)]			
Light ATPase Fibers	12.870 ± 0.419 (164)	5.673 ± 0.246 (184)	9.463 ± 0.399 (152)
Dark ATPase Fibers	12.500 ± 1.256 (20)	7.774 ± 0.766 (29)	12.817 ± 1.039 (41)

Values are means ± standard error of the means, based on 4, 6, and 5 rats in the CON, HS, and HS-EX, respectively. The number of fibers analyzed for each assay is given in parentheses.

0.05) mean SDH activity than the light ATPase fibers; i.e., 40, 24, and 29% higher in the CON, HS, and HS-EX groups, respectively (Table II).

The total number of enzyme units in a fiber—i.e., the enzyme activity normalized by the area of the fiber (integrated activity (I) = enzyme activity (OD/min) × CSA (μm^2))—was determined to account, in part, for the differential changes in CSA accompanying the suspension and exercise procedures. In comparison to CON, mean ISDH values decreased by 56 and 38% ($p > 0.05$) in light and dark ATPase fibers of HS rats, respectively (Table II). In comparison to the HS group, treadmill exercise increased the total number of enzyme units by approximately 65% ($p > 0.05$) in both fiber types. Further, the mean ISDH in the dark ATPase fibers was only slightly higher than CON (Table II), while the mean SDH activity was 40% higher. In HS rats, there was a general shift towards lower values for both fiber types (Fig. 4). In the HS-EX rats, the range of ISDH values for both fiber types was similar to CON. However, in the light ATPase fibers of HS-EX rats, there was a higher proportion of fibers in the lower range of values, while in the dark ATPase fibers a higher proportion of fibers were in the upper range of values (Fig. 4).

DISCUSSION

Body, muscle, and adrenal weights: The soleus mass was decreased to ~30% of CON after 28 d of HS (Table I). These data are consistent with the results of previous HS studies that report a dramatic loss in muscle mass in a relatively short period of time using a variety of suspension procedures and time intervals. For example, the magnitude of loss of soleus mass appears to be greatest within the first week followed by a slower, progressive loss over months; i.e., 7 (5), 20 (5), 30–45 (5,32), 40–50 (6,17,31,32), 40–70 (11,12,13,17,22,31,32,35, present data) and 55% (17) decreases in soleus mass after 3, 5, 7, 14, 28 and 90 days, respectively. Spaceflight also results in a rapid decrement in soleus mass. For example, Grindeland *et al.* (10) reported a 24 and 36% decrease in the soleus mass of large and small rats, respectively, after 7 d of spaceflight, while Ilyina-Kakueva *et al.* (15) reported a 32% decrease after a 20-d Cosmos flight.

The decrease in muscle weight associated with HS could be due, in part, to a decrease in the total body growth rate. Some studies have reported a decreased growth rate of suspended rats based on body weight (7,17,28), whereas others have not (1,11,12,24,31,32, 33). Although the reasons for these discrepancies are unknown, one plausible explanation could be related to the amount of stress placed on the rats by the method of suspension. In the present study, the average adrenal weights were similar in the CON and HS group (Table I), suggesting a minimal level of stress imposed by our suspension procedures. In addition, the mean body weight of the HS group was only 10% ($p < 0.05$) lower than CON (Table I). Thus, the relative muscle weight (i.e., the muscle weight/body weight ratio) would be decreased in proportion to the absolute muscle weight, indicating that the atrophy is occurring primarily in the muscle. Similar conclusions have been drawn by Flynn *et al.* (7) who report decreases in the soleus wet weight/body weight ratios following HS. Together, these results suggest that the smaller muscle weights in HS rats are not entirely due to a lower body weight.

Other factors to consider in the interpretation of the smaller muscle masses associated with HS are the rates of protein synthesis and degradation. Jaspers and Tischler (16) report a slower rate of protein synthesis and an increased rate of protein degradation in the soleus following HS. Increases in the excretion of 3-methylhistidine and urea and in protease activity (31), markers of protein catabolism, have been shown after HS. Steffen and Musacchia (29) report a lower absolute RNA level in the soleus of HS rats, suggesting a reduction in the rate of protein synthesis. We did not measure protein metabolic rates in the present study. However, histological examinations of the muscle cross sections revealed a number of indicators of fiber degeneration; e.g., a small percentage of fibers were irregularly shaped and/or ragged, had central vacuolations, stained abnormally intense for the metabolic enzymes, etc. (data not shown). These histological abnormalities have been observed and discussed previously by other investigators following both HS (31) and spaceflight (23). No fibers showing these abnormalities were included in our quantitative data analyses.

Fiber size and type: The reduction in cross-sectional area of the light ATPase fibers paralleled the loss in muscle mass (i.e., ~70%), while the reduction in the size of the dark ATPase fibers was smaller (i.e., ~45%). However, most of the atrophy occurred in the light ATPase fibers since this type of fiber makes up the largest proportion (~90% in the control rats) of the mus-

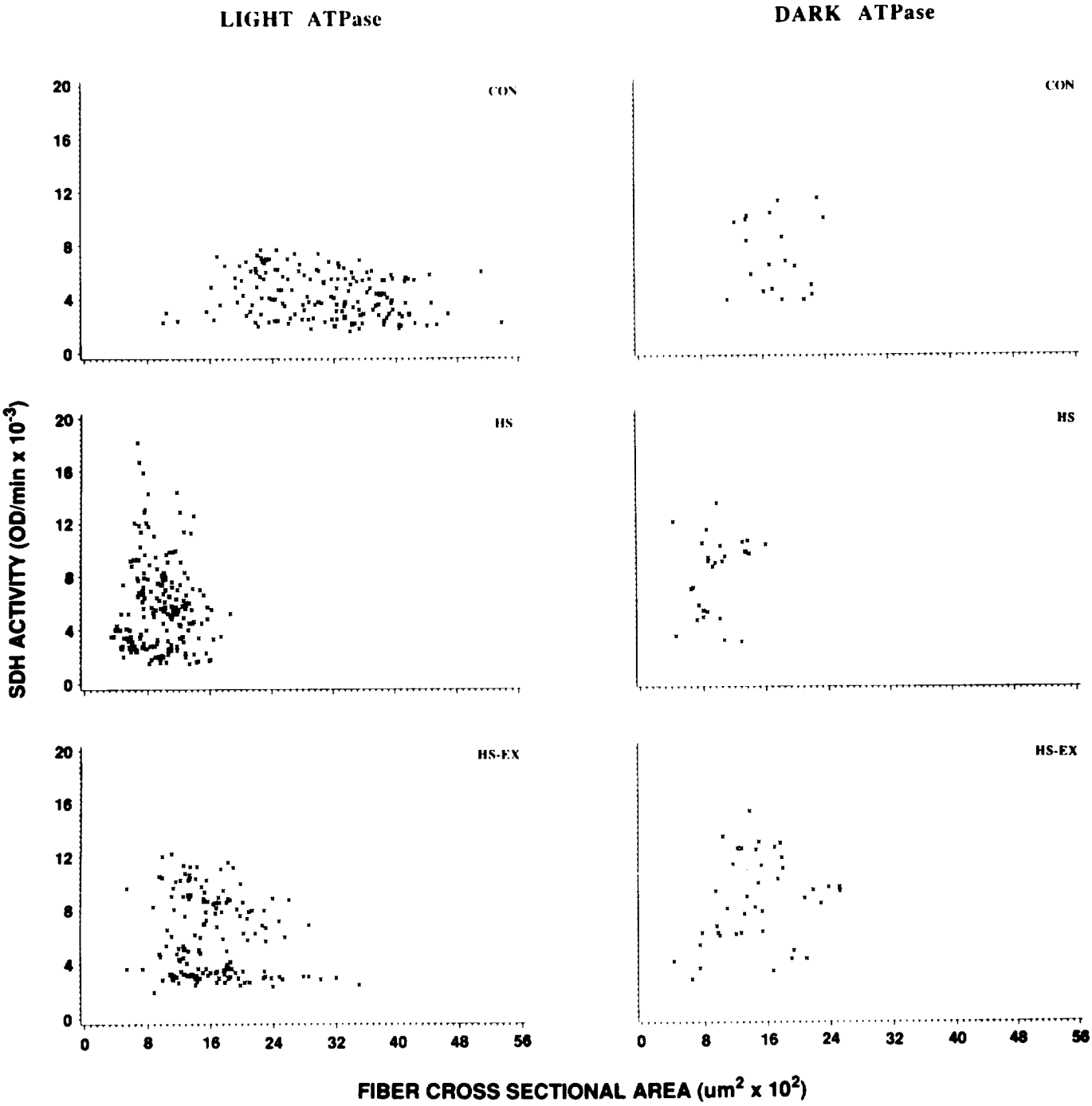

Fig. 3. Relationship between succinate dehydrogenase (SDH) activity and fiber cross-sectional areas for light and dark ATPase fibers of the soleus of control (CON), 28-d hindlimb suspended (HS), and HS plus exercise (HS-EX) rats.

cle. Differential atrophy in the two fiber types has been reported previously. In general, it appears that the larger, light ATPase fibers of the soleus (11,12,31), as well as the relatively small light ATPase fibers in mixed fast muscles (24), are more susceptible to the removal of their load-bearing function than the dark ATPase fibers. It is of interest also that the size of the fibers tends to reduce to a similar size suggesting that there may be some minimum size that is optimal for an unloaded muscle. This trend has been observed in several studies (11,31).

The percent dark ATPase fibers doubled in the HS rats in comparison to CON, a finding consistent with previous histochemical (11,12,28,31) and biochemical (32,33) reports. These adaptations in myosin ATPase profile have been linked to the increases in the contractile speed properties of HS soleus muscles (6,31,35).

Single fiber SDH activities: SDH activity was 40% higher in HS rats compared to CON in the light ATPase fibers and was relatively unchanged (i.e., an 11% difference) in the dark ATPase fibers (Table II). Using the same quantitative histochemical procedures described in the present study, Hauschka *et al.* (11) observed a higher SDH activity of light ATPase fibers after 28 d of

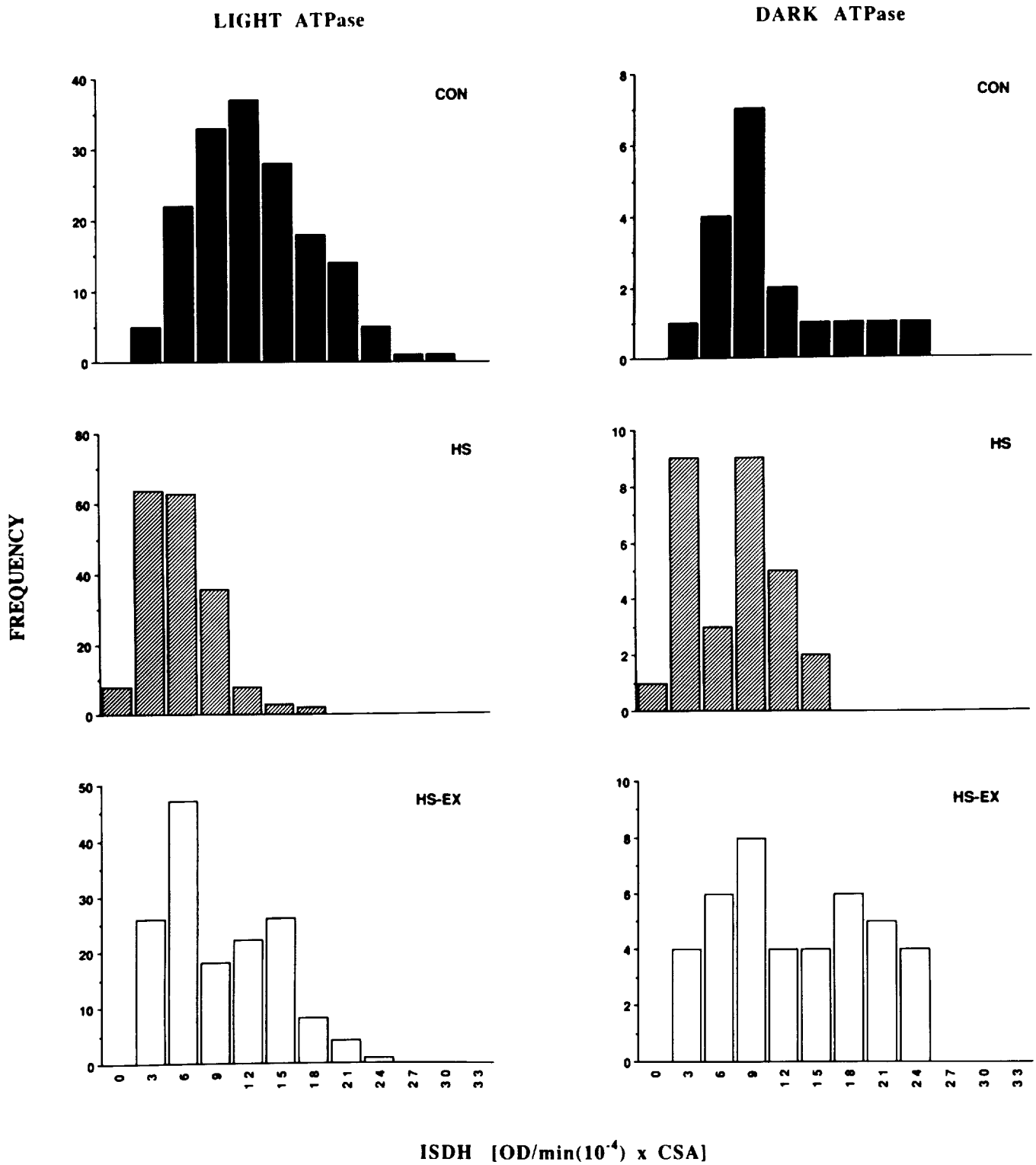

Fig. 4. Frequency distributions of integrated succinate dehydrogenase (ISDH) activity (activity/min × CSA) for light and dark ATPase fibers of the soleus of control (CON), and 28-d hindlimb suspended (HS) and HS plus exercise (HS-EX) rats.

suspension, and Martin *et al.* (19) showed a maintenance of SDH activity in comparison to gound-based CON rats in both fiber types following a 7-d spaceflight. In contrast, there have been reports that the oxidative capacity of the soleus muscle is decreased following HS (4,7,28) and spaceflight (15,23). However, these latter reports are based on whole muscle homogenates, qualitative histochemical techniques and electron microscopy. These discrepancies may be due, at least in part, to the differences between the analysis procedures. For example, whole muscle homogenates include noncontractile tissues in the muscle mass, e.g., connective tissue. Based on previous data (7) and unpublished observations in our animals (A. Vailas, personal commu-

nication), it appears that there is an increased concentration of connective tissue in HS rats. The presence of these non-muscle tissues would result in a dilution of the enzyme activities in whole muscle homogenates. The technique used in our analysis avoids this potential problem by quantifying the amount of reaction product formed during a given unit of time within the defined borders of a fiber.

In the present study the fiber enzyme activity was assessed as a function of fiber size. If a fiber atrophied without any concomitant absolute decrease in the number of enzyme units, then the enzyme activity would increase in proportion to the decrease in fiber size. To estimate the extent that this interactive effect may have occurred in this study, integrated optical density (I) values were calculated by multiplying the SDH activity times the area of the muscle fiber. This value indicates the relative number of enzyme units per fiber, assuming all SDH enzyme units have the same specific activity. Since the ISDH activity per fiber decreased in the HS group (Table II), there would have been a net loss of the SDH enzyme per fiber assuming no change in specific activity.

Since the SDH activity of the fiber is, at the least, maintained and in some cases increased after HS, it might be expected that the fibers would maintain their high resistance to fatigue. In fact, a maintenance of fatigue resistance of the soleus has been reported following 7 d (7,13,22) and 28 d (35) of HS. Similarly, fatigue resistance in the soleus of cats is maintained following prolonged periods of reduced neuromuscular activity; e.g., 6–12 months after spinal cord transection (2) or spinal isolation (26).

Effects of endurance exercise: Treadmill running was used in an effort to ameliorate the atrophic and metabolic changes associated with HS. The HS-EX group did exhibit several improvements over the HS group (Tables I and II). Both body weight and soleus wet weight were significantly higher in the HS-EX than the HS group, suggesting that exercise ameliorated, but did not prevent, the atrophic response to unloading. Similarly, Sullenger *et al.* (30) recently reported that 15 or 120 min of treadmill running at 13 $m \cdot min^{-1}$ and 0% grade for 5 d per week resulted in only a 32% decrease in soleus mass after 28 d of HS, as compared to a 49% decrease in the unexercised group. While these data suggest that periodic loading of the soleus during hindlimb suspension decreases the magnitude of the atrophy, they also show that similar effects can be obtained from a wide range of loading durations, e.g., 15–120 min.

The exercise protocol used in the present study, in conjunction with HS, apparently was very stressful to the rats, since the adrenal weights of the HS-EX group were 52% higher than either the CON or HS groups (Table I). It is known that glucocorticoids released under stressful conditions act directly on skeletal muscle, enhancing protein catabolism (8,25). In spite of this added stress, the soleus muscles of the HS-EX group were 130% larger than the HS group, suggesting that the treadmill exercise was a potent stimulus for maintaining muscle mass. In comparison to the HS rats, the mean cross-sectional area of both fiber types in the HS-EX group were closer to CON values, with the dark ATPase fibers being only 18% smaller than CON (Fig. 1). These data suggest that the dark ATPase fibers were more responsive to this form of exercise.

The treadmill exercise protocol used has been shown to induce significant increases in the oxidative capacity of the involved skeletal musculature in normal rats (14). In the present study, the exercise ameliorated several of the metabolic-associated changes resulting from HS. The soleus fibers of HS-EX rats had elevated SDH activity compared to HS rats (Table II). Further, the number of enzyme units per fiber in the HS-EX was similar to CON in the dark ATPase fibers, and only 26% less than CON in the light fibers. Similarly, the ISDH in the HS-EX was about 65% higher than HS in both light and dark ATPase fibers.

In summary, it appears that the daily treadmill exercise ameliorated the atrophic response in the light and dark ATPase fibers of the hindlimb suspended rat soleus. Cross sectional areas of the light ATPase fibers were reduced to a greater degree than the dark ATPase fibers during hindlimb suspension, while the exercise appeared to prevent atrophy to a greater extent in the dark ATPase fibers. SDH activity was enhanced somewhat by the suspension protocol in both fiber types, with a greater increase in the light ATPase fibers. Daily exercise resulted in a further increase in the SDH activity of only the dark ATPase fibers. In addition, the increase in SDH activity generally resulted from a relatively greater reduction in cross sectional area than in SDH enzyme units as reflected by the changes in ISDH values.

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PULMONARY FUNCTION IN MICROGRAVITY: SPACELAB 4 AND BEYOND†

H. J. GUY,‡ G. K. PRISK§ and J. B. WEST¶
Department of Medicine M-023A, University of California—San Diego, La Jolla, CA 92093, U.S.A.

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Abstract—The paper refers principally to the composition gradient of gases within the lung in various conditions of gravity, as revealed by exhaled breath. A rapid gas analyzer-based system has been developed for tests in Spacelab 4. The test sequence and expected results are presented.

1. INTRODUCTION

The large apico-basal gradients in alveolar size, ventilation, capillary distension and blood flow that exist in the erect human lung cause readily measurable gradients in regional lung function[1]. There are also gradients in the ratio of airflow ($\dot{V}_A$) and bloodflow ($\dot{Q}_c$), and thus in gas exchange and local alveolar gas concentration. Gradients in gas concentration also occur when a foreign gas is inhaled, because the ventilation per unit volume differs topographically[2].

These gradients in gas concentration can be seen during single, slow vital capacity exhalations, when we use a rapidly responding gas analyzer to sample gas concentrations at the lips. The heart causes pulsatile flow from the dependent lung regions, and thus there are "cardiogenic" oscillations in CO_2 and O_2 concentration due to the topographic gradients in gas exchange. Similarly there are oscillations of N_2 (the resident gas) if a breath of a diluent foreign gas has just been inhaled[3]. The lungs static mechanical properties cause the dependent regions to stop emptying first during exhalation, and this can be seen as a terminal inflection in gas concentration as flow from the upper regions predominates[4].

We can, therefore, study gravitational gas concentration gradients quickly and non-invasively. Michels and West[5] of our group did this in a NASA LearJet, which was flown through 30 second Keplerian arcs. Cardiogenic oscillations and terminal inflections in gas concentration virtually disappeared at zero-*g* confirming that the topographic gradients are gravitationally induced (Fig. 1).

We will soon be repeating the LearJet tests in Spacelab 4 (SL-4), along with a more extensive package of tests to not only study influences of topographic gradients in lung function, but also to study the overall function of the lung in the presence of the headward redistribution of blood and liquid that occurs at the onset of exposure to microgravity. We will perform the test battery repeatedly preflight and postflight in both the supine and erect posture and at intervals thoughout the SL-4 mission to study adaptation to the space environment, and the consequences of that adaptation postflight.

We describe here the details of the SL-4 experiment, and some of the more interesting predictions that we can make about the results we will obtain.

2. METHODS

2.1. Hardware

Single breath testing is almost ideally suited to the Spacelab environment. A large amount of information can be otained by analysis of instantaneous gas concentration and flow at the lips, as the subject switches from breathing cabin air, to the inhalation and exhalation of a series of test gas mixtures[6].

The primary requirement is for a rapidly responding gas analyzer. NASA life sciences provide a Gas Analyzer Mass Spectrometer (GAMS) as a shared instrument. We use it to measure O_2, CO_2, N_2, AR, $C^{18}O$ and N_2O. It samples from the mouthpiece of our equipment at 60 ml/min and has a 10–90% response time of approx. 100 ms.

We have developed a rack mounted system, composed of a "bag-in-box" assembly, an electronics control assembly and a gas cylinder assembly (Fig. 2). The bag-in-box assembly allows the subject to be switched from breathing cabin air, to a series of premixed gases in separate breathing bags. These are enclosed in a rigid box, which is open to the cabin via a flowmeter (Fleisch No. 2 pneumotachograph) that maintains a continuous recording of respiratory flow throughout the test sequence.

The bag-in-box assembly is supported by the electronics controlling assembly (ECA). this allows inter-

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‡M.D.
§Ph.D.
¶M.D., Ph.D.
‖Academy Member, Section 3; Life Sciences

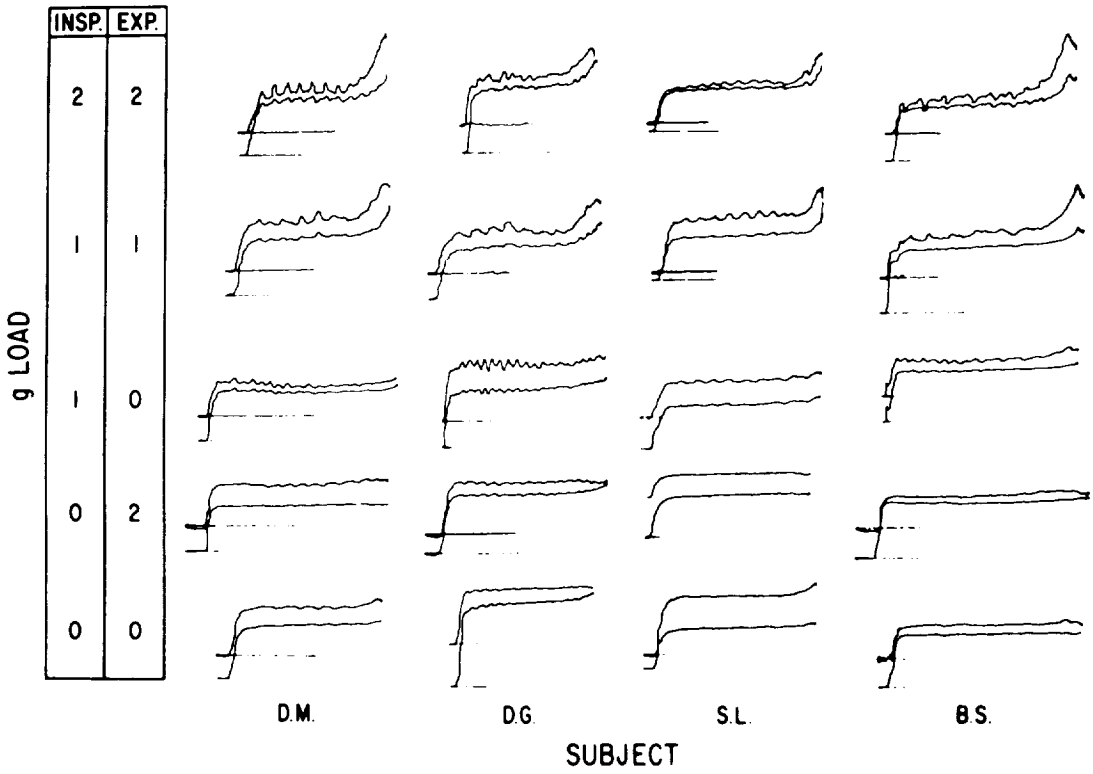

Fig. 1. Comparison of single breath washouts from four subjects (columns) at differing inspiratory and expiratory g loads (rows). Each subject inhaled a 150 ml bolus of argon, followed by pure O_2 to vital capacity just prior to the exhalations. Argon concentrations (upper traces) and nitrogen concentrations (lower traces) are plotted. Note the cardiogenic oscillations and terminal concentration rises: they are increased at 2-g, and markedly reduced at 0-g. From Michels and West[5].

action between the subject and a dedicated NASA microcomputer via an alphanumeric display and keypad. The ECA transmits analog data to the microcomputer, which in turn sends 14 channels of 12 bit A/D logged at 160 Hz, into the Spacelab data system. The system controls the subject's tidal volume, breathold times and flowrates, by activation of solenoid valves. Tidal volume control requires precise linearization of the flowmeter using a 6809 microprocessor lookup table[7]. This limits drift of the generated volume signal to 20 ml during a 20 breath nitrogen washout.

The bag-in-box system is also interfaced to the gas cylinder assembly which dispenses the premixed gases (Table 1), and to the spacecraft vacuum system which enables bag emptying on computer command.

2.2. The test sequence

Syringe calibration of the integrating flowmeter is followed by automated sampling of all stored gas mixtures and the internal GAMS calibration gases. This is followed by a fixed test sequence that has been developed to minimize interaction and maximize efficiency[6] and is now finalized as in Table 1.

A period of quiet breathing on the mouthpiece is recorded allowing analysis of resting ventilation and gas exchange. This is followed by a standard single breath nitrogen (SBN) washout, in which the subject inhales a 150 ml argon bolus at the start of a vital capacity breath of oxygen, then immediately exhales though a newly developed flow regulator at 0.5 l/s.

The subject then performs a hyperventilation-breathold maneuver to maximize any gradients in the lung due to perfusion inhomogeneity, and then exhales at 0.5 l/s[5]. A standard single breath CO diffusing capacity (D_LCO) measurement follows[8] and is in turn followed by a period of oxygen breathing (multibreath nitrogen washout), in which the tidal volume and lung volume are rigidly controlled by solenoid valves[9]. A second D_LCO is then performed with the hyperoxic $C^{18}O$ mixture, allowing calculation of the pulmonary capillary blood volume (V_c)[8].

A period of quiet cabin air breathing follows. Data collected during this period are used to define the parameters of a single compartment model of gas exchange. A vital capacity 0.5 l/s exhalation is then recorded, allowing analysis of intrabreath gradients in the respiratory exchange ratio (R), and thus of gradients in gas exchange, with a correction for continuing gas exchange using the model[6].

Rebreathing of the N_2O containing mixture follows. Data are analyzed to obtain cardiac output and lung volumes[10,11]. Finally, repeated forced ex-

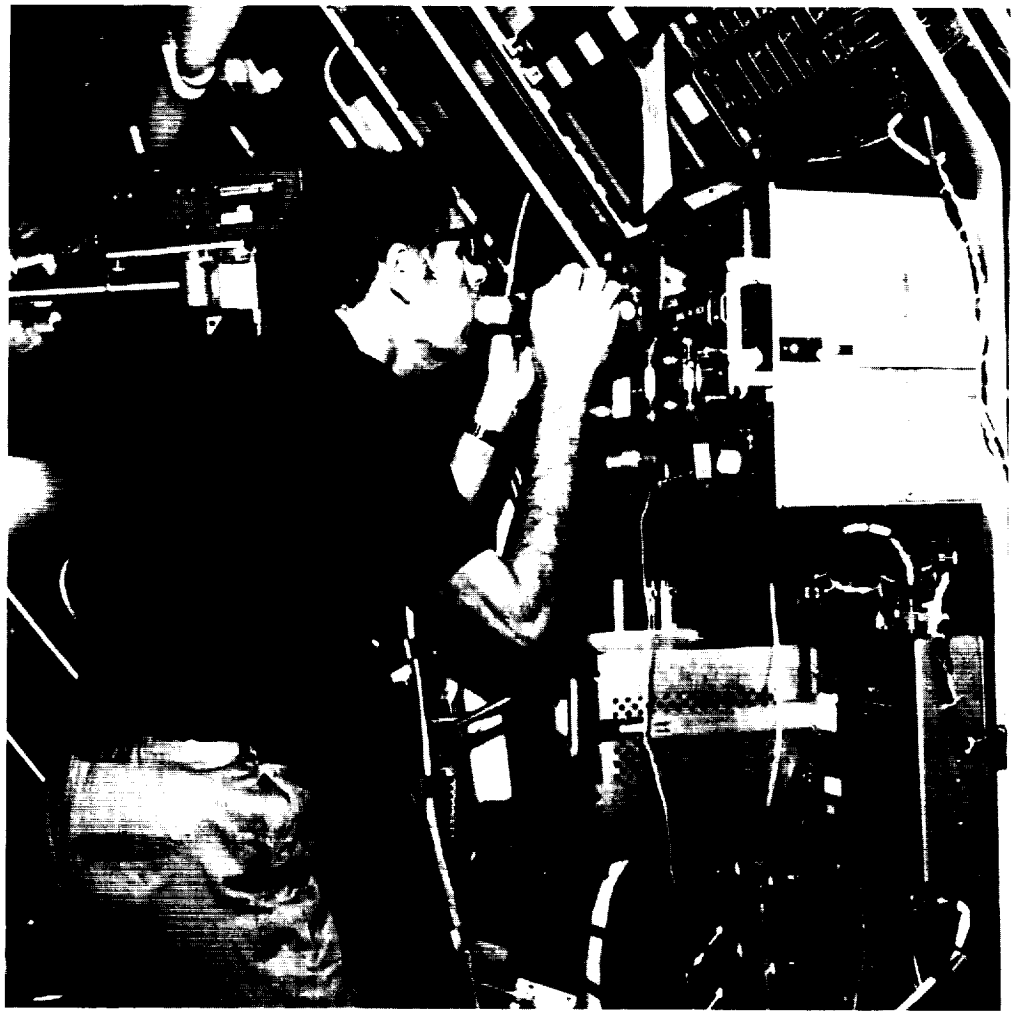

Fig. 2. Robert W. Phillips D.V.M., Ph.D., SL-4 payload specialist, using the rack-mounted experiment hardware. He is following prompts displayed on the electronics control assembly (ECA) above the deployed bag-in-box assembly. A mouthpiece probe is connected to the mass spectrometer below the bag-in-box.

piratory spirometry is performed. Data will be analyzed to define any changes from the characteristic 1-g flow/volume plot that is seen in each subject, in several gravitational orientations[12].

3. PREDICTIONS AND HYPOTHESES

3.1. Uneven ventilation

We expect the cardiogenic oscillations and terminal rises in the SBN argon and nitrogen traces to virtually disappear. Any residual oscillations or terminal inflections will be of great interest (in the LearJet experiment they were less significant because the lung had been deformed by accelerations immediately before entry into ballistic flight). Small cardiogenic oscillations may also persist because of lobar differences in specific ventilation. The terminal nitrogen rise may persist in part. It can be shown to be due to a different mechanism in abnormal subjects[13], that is non-gravitational[14] and in the gravity free environment of Spacelab, a similar small non-gravitational signal may well emerge in normals.

The LearJet experiment showed that the SBN alveolar plateau slope persisted at 0-g although gravitational inhomogeneity was long thought to be its major cause. It is now thought to be largely due to intra-acinar diffusive/convective inhomogeneity, a consequence of asymmetry of branching at the alveolar duct level[15]. In the gravity-free environment the small gravitational component may become definable.

Analysis of the multibreath nitrogen (MBN) test will be particularly interesting. It has recently been shown that the normalized alveolar slope progressively increases from breath to breath. The rapid increase in slope in the first few breaths is a measure of the intra-acinar diffusive inhomogeneity, as evidenced by He/SF_6 studies and modeling[16]. The

Table 1

Test	Abbrev.	Gas mixtures	Reference
Resting gas exchange	RGE	Air	
Single breath N_2 washout	SBN	(a) Oxygen (b) 79% Argon 21% O_2	[5]
Distribution of perfusion	QDT	Air	[5]
Single breath diffusion test	DCO	0.3% $C^{18}O$ 10% Ar 21% O_2 Balance N_2	[18]
20 breath N_2 washout Followed by: hyperoxic diffusion (V_c) test	MBN	Oxygen 0.3% $C^{18}O$ 10% Ar Balance O_2	[9] [8]
$\dot{V}_A/\dot{Q}_c$ test	VQD	Air	[6]
Rebreathing cardiac output	REB	2% N_2O 10% Ar 21% O_2 Balance N_2	[10] [11]
Spirometry	SPI	Air	[12]

increasing slope beyond the fifth breath depends on convective inhomogenity at a grosser level. We can predict that the initial rate of change will persist on orbit. The subsequent rate of change will give new insights into the relative contributions of gravitational and non-gravitational mechanisms to convective inhomogeneity in the lung.

We can predict that the distribution of specific ventilation[9] obtained from the MBN data at 0-***g***, will be tighter than at 1-**g**. We can also predict that the SBN data and MBN data will be re-analyzed in new ways for many years, and will remain a valuable resource.

3.2. Perfusion

As in the LearJet experiment, we expect to see a big reduction in oscillations of O_2 and CO_2 concentrations. The Intrabreath R tracing should also show greatly reduced oscillations. The terminal rise in R seen in normal subjects, differs from the terminal fall, seen in subjects with airflow limitation[6]. It may be that a small fall in R, beyond that explicable by continuing gas exchange, is seen at 0-***g***. This would be evidence of the presence of a population of poorly ventilated alveoli with maintained perfusion; early evidence of lung "aging" that cannot be obtained in the presence of gravity. The persistence of small cardiogenic oscillations might suggest residual lobar perfusion differences.

Postflight, in the erect position, there may be very large gravitational gradients in blood flow. These would have some operational significance, as one could predict that gradients during re-entry would also be large[17]. We do not yet know if adaptation to 0-***g*** causes an increase in post exposure gravitational gradients in the lung.

3.3. Pulmonary capillary volume and blood flow

We can predict that the changes seen in immersion[18] and bedrest[19] will occur. The cardiac output, D_L CO and its V_c component should increase on orbit. The extent of the increase and its persistence is not currently predictable, but it seems likely that the bedrest model is more realistic than the earlier immersion model, and changes will not persist after cardiovascular/renal adaptation. Postflight erect/supine differences may well be larger than those seen preflight.

3.4. Forced expiratory spirometry

When plots of instantaneous flow are made on an expired volume plot during maximum exhalation, individual sujects have a characteristic curve that has definite shape, and usually, very definite "bumps" that are reproducible. This characteristic "MEFV" curve differs as posture is changed[12]. It is thought that maximum flow is determined by wave speed of deflection of bronchial walls in a critical flow limiting segment. This segment moves from the trachea to the peripheral airways as lung volume diminishes. The supine MEFV curve shows significantly greater flow at large lung volumes, and interestingly, decreased flow at low lung volumes. It was predicted from current theory that gravitational inhomogeneity of airway pressure/area relationships should cause the opposite change at low lung volumes[12]. The decreased flow at low lung volumes, in the supine position, might be due to vascular/liquid cuffs around the small airways. If this also occurs at 0-***g*** and especially if it returns toward normal with time on orbit, the vascular/liquid cuff theory will be supported.

4. CONCLUSIONS

For many years gravitational gradients in lung function have been recognized and studied. These gradients have made it difficult to study non-gravitational inhomogeneity in normal subjects. The development of a microgravity pulmonary laboratory, therefore, should provide a major resource for those who study the normal lung, both in the immediate and more distant future.

There are many practical applications that will also be of interest. While experience suggests that congestion of the lung with blood does not pose a problem at rest, there is less certainty that it is not a problem under severe exercise conditions, soon after the onset of weightlessness. There is also the possibility that inhalation lung injury (from aerosols, gases, and vapors) can occur in a closed 0-***g*** environment. Aerosol deposition in the lung itself at 0-***g***, warrants study as sedimentation will no longer occur. Measured differences in deposition will not only be of practical, but also of great theoretical interest. High oxygen atmospheres that are required for pre-EVA

denitrogenation can exacerbate the effects of any pre-existing pathology on gas-exchange[20]. Total body denitrogentation itself is simply an extended "MBN test" and can be quantitated and compared with denitrogenation on the ground, from which it should differ because of the redistribution of bloodflow in the tissues of the body. We expect many groups to share this exciting initial exploration of pulmonary function in microgravity.

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